Expanded View Figures

Figure EV1. The IFNβ response to dsDNA is length-dependent at low DNA concentration (related to Fig 1A–E).

A Size specificity of PCR-derived dsDNA ranging from 88 bp to 4,003 bp was confirmed by high-sensitivity automated gel electrophoresis (Fragment Analyzer). The 6,000 bp indicates the upper marker.

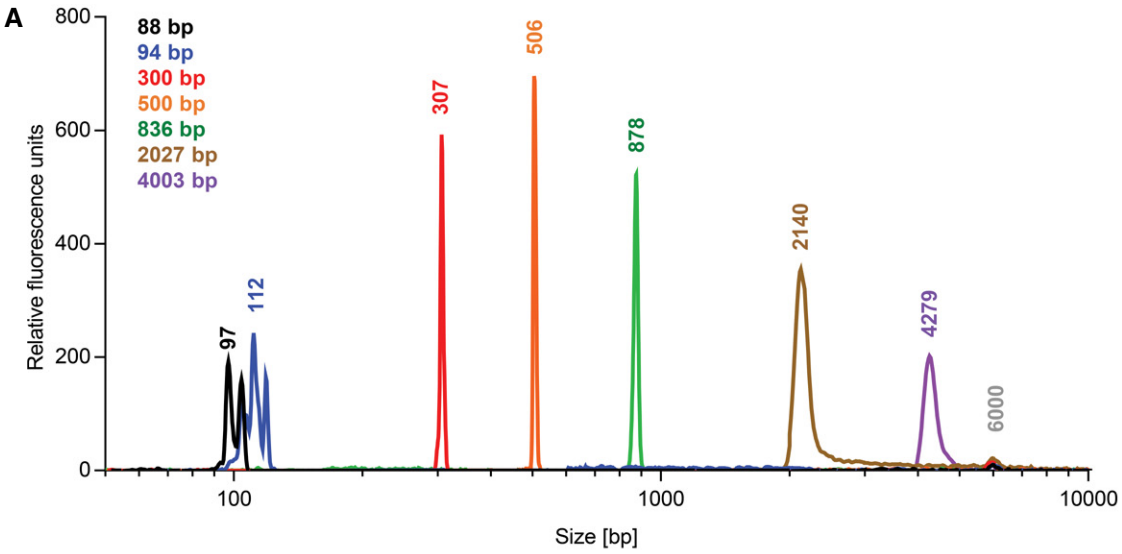
B 200 ng of the PCR-derived DNA stocks (100 ng/μl) used for transfection of cells was visualized by agarose gel electrophoresis.

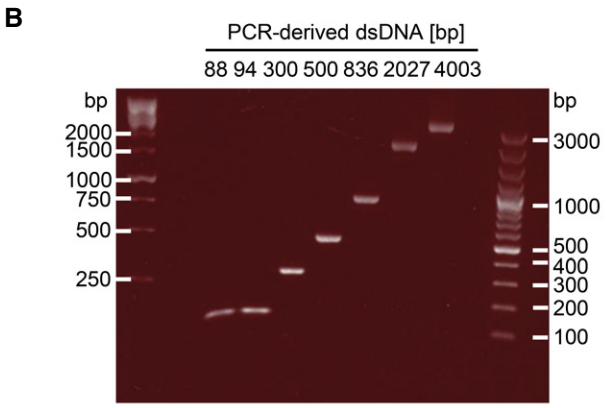
C IFNβ mRNA levels (normalized to β-actin mRNA levels) in PMA-differentiated THP-1 cells transfected with PCR-derived dsDNA of indicated lengths at 1.67 μg/ml or 0.167 μg/ml for 6 h, measured with qPCR.

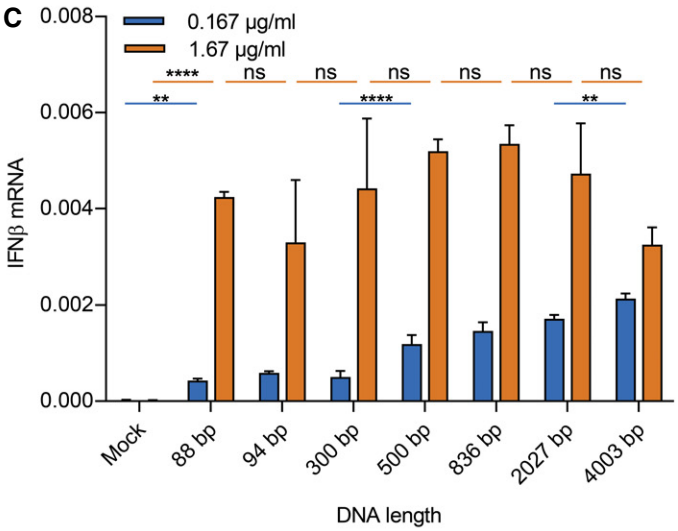
D IFNβ mRNA levels (normalized to β-actin mRNA levels) in PMA-differentiated THP-1 cells transfected with PCR-derived dsDNA of indicated lengths at 0.0167 μg/ml for 6 h, measured with qPCR.

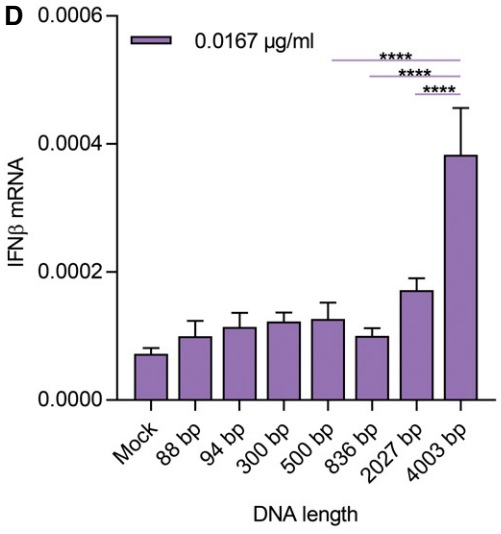
E Type I IFN levels in supernatants from PMA-differentiated THP-1 cells transfected with Lipofectamine RNAiMAX with PCR-derived dsDNA of indicated lengths at 0.167 μg/ml for 12 h, measured by bioassay. The broken horizontal line indicates detection limit of assay.

Data information: Data are represented as mean ± SD of biological triplicates in one experiment. Statistical significance was analyzed using one-way ANOVA. ns non-significant, $^{**}P < 0.01$, $^{****}P < 0.0001$. The experiments were performed three times.


A
88 bp
94 bp
300 bp
500 bp
836 bp
2027 bp
4003 bp
Relative fluorescence units
Size [bp]
97
112
307
506
878
2140
4279
6000


B
PCR-derived dsDNA [bp]
88 94 300 500 836 2027 4003
bp
2000
1500
1000
750
500
250
3000
1000
500
400
300
200
100


C
0.167 µg/ml
1.67 µg/ml
IFNβ mRNA
DNA length
Mock
88 bp
94 bp
300 bp
500 bp
836 bp
2027 bp
4003 bp


D
0.0167 µg/ml
IFNβ mRNA
DNA length

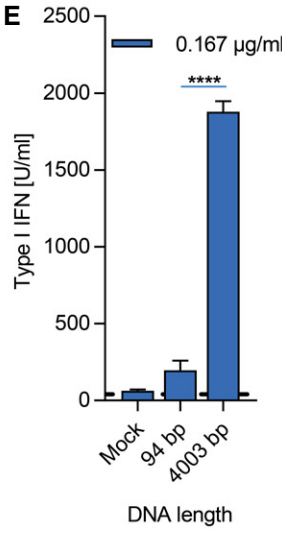

E
0.167 µg/ml
Type I IFN [U/ml]
DNA length

Figure EV1.

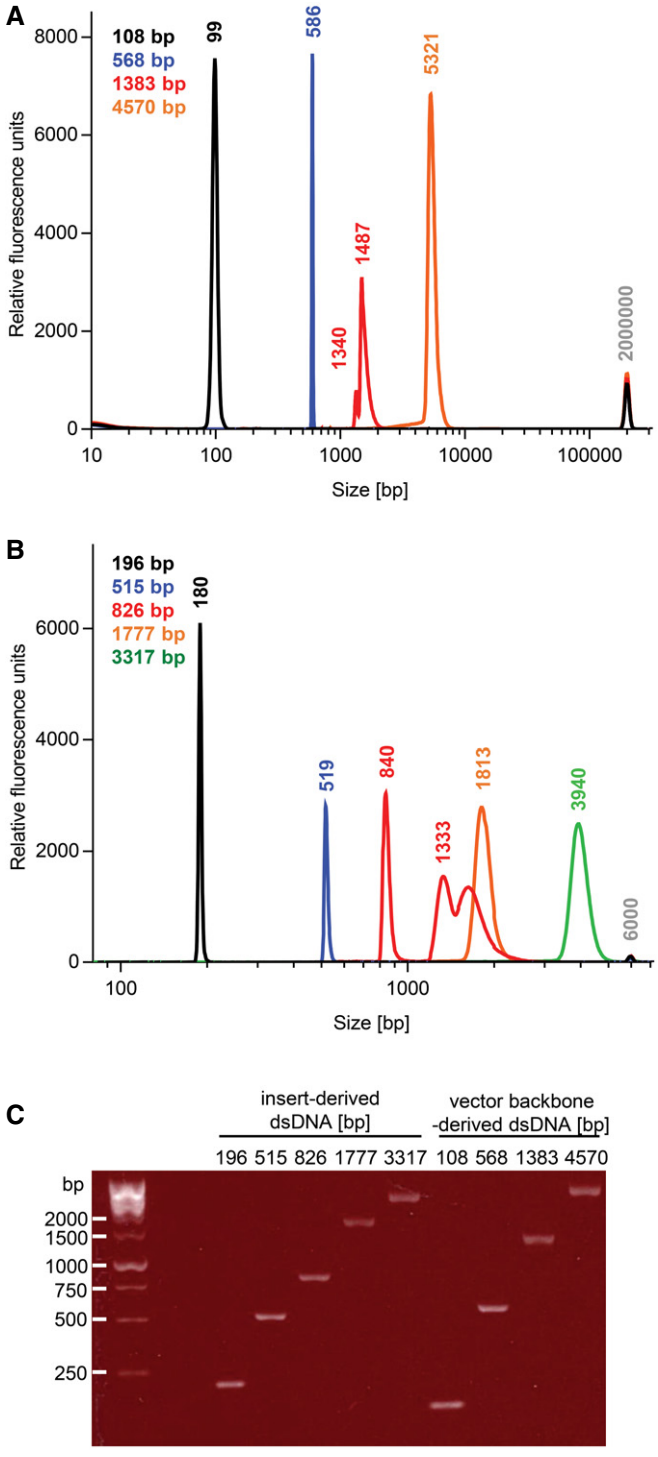


Figure EV2. Restriction digestion-derived dsDNA for transfection of THP-1 cells (related to Fig 1F and G).

A Size specificity of plasmid backbone-derived restriction fragments ranging from 108 to 4,570 bp was confirmed by high-sensitivity automated gel electrophoresis (Fragment Analyzer). The 200,000 bp indicates the upper marker.

B Size specificity of human gene insert-derived restriction fragments ranging from 196 to 3,317 bp was confirmed by high-sensitivity automated gel electrophoresis (Fragment Analyzer). The 6,000 bp indicates the upper marker. The 826-bp fragments show some contamination with a slightly larger DNA sized at 1,333 bp, which is not visible by agarose gel electrophoresis (see panel C).

C 100 ng of the stocks of restriction fragments (OAS3 insert-derived and plasmid backbone-derived) (30 ng/µl) used for transfection of cells was visualized by agarose gel electrophoresis.

Figure EV3. Length-dependent sensing of transfected dsDNA is dependent on the cGAS-STING pathway (related to Fig 2).

A cGAS, STING, IFI16, TREX1, and vinculin protein levels in lysates of cGAS-, STING-, IFI16-, and TREX1-deficient PMA-differentiated THP-1 cells were determined by Western blotting after SDS–PAGE.

B IFNβ mRNA levels (normalized to β-actin mRNA levels) in PMA-differentiated THP-1 cells with a deletion in STING or in an unrelated gene (Control) transfected with PCR-derived dsDNA of indicated lengths at 0.167 μg/ml for 6 h, measured with qPCR.

C IFNβ mRNA levels (normalized to β-actin mRNA levels) in PMA-differentiated THP-1 cells with a deletion in cGAS or in an unrelated gene (Control) transfected with PCR-derived dsDNA of indicated lengths at 0.167 μg/ml for 6 h, measured with qPCR.

D, E Levels of phosphorylated TBK1 (p-TBK1) (D) and dimerized STING protein (E) in PMA-differentiated THP-1 transfected with PCR-derived dsDNA of indicated lengths at 1.67 μg/ml for 2 h. Proteins in cell lysate were separated by SDS–PAGE under reducing (D) or non-reducing (E) conditions. After Western blotting, membranes were probed with anti-TBK1, anti-pTBK1 (D), anti-STING (E), and anti-vinculin as a loading control.

F cGAMP levels in PMA-differentiated THP-1 cells transfected with PCR-derived dsDNA of indicated lengths at 1.67 μg/ml for 8 h, determined by semi-quantitative LC-MS/MS analysis.

Data information: Data are represented as mean ± SD of biological triplicates in one experiment. Statistical significance was analyzed using one-way ANOVA. ns non-significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. The experiments were performed three times (B–E) or two times (F).
Source data are available online for this figure.

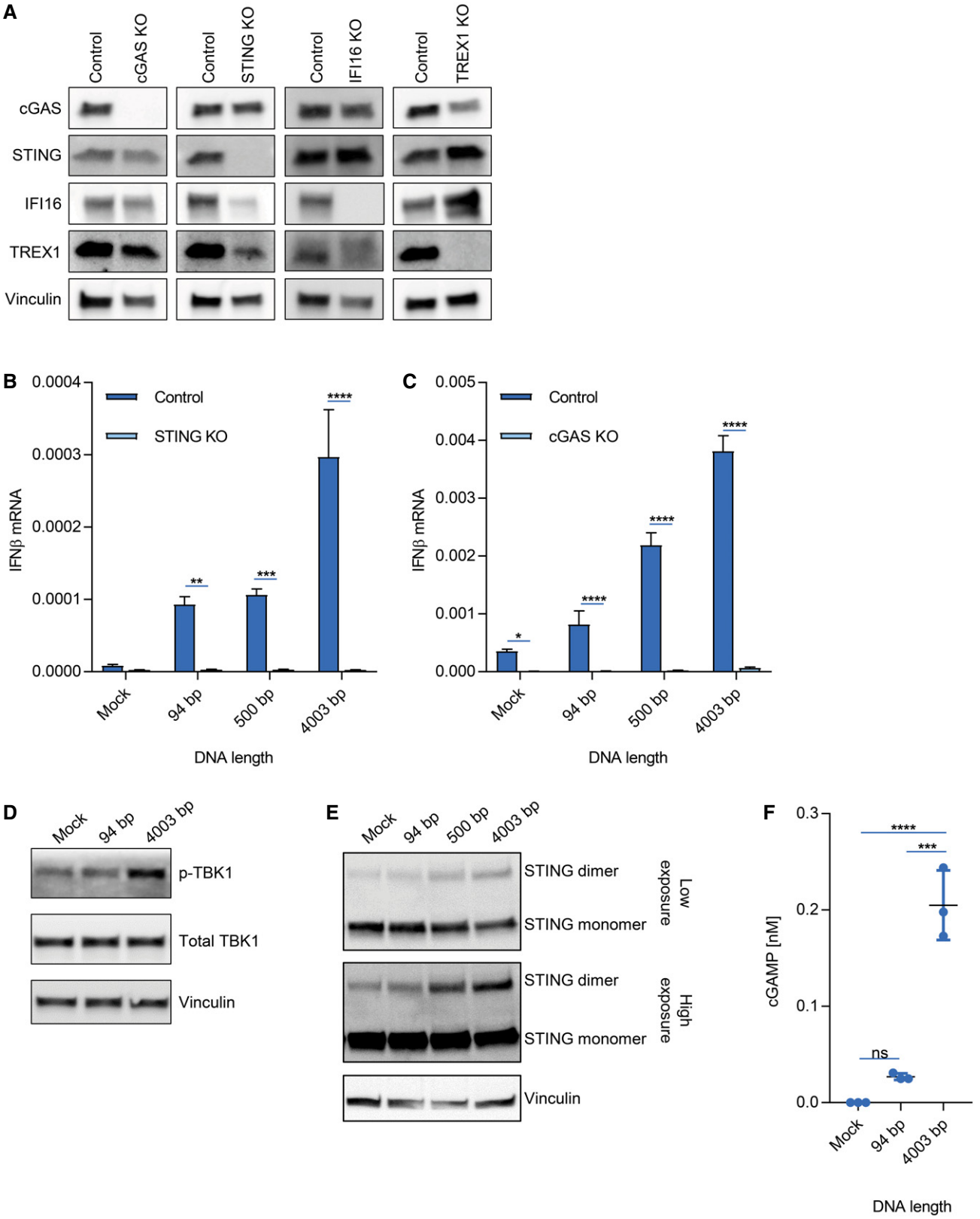


Figure EV3.

Figure EV4. The length-dependent stimulation of cGAMP production by DNA is explained by cGAS alone (related to Fig 3).

A IFNβ mRNA levels (normalized to β-actin mRNA levels) in PMA-differentiated THP-1 cells with a deletion in TREX1 or control cells transfected with PCR-derived dsDNA of indicated lengths at 0.033 μg/ml for 6 h, measured with qPCR.

B IFNβ mRNA levels (normalized to β-actin mRNA levels) in PMA-differentiated THP-1 cells with a deletion in IFI16 or in an unrelated gene (Control) transfected with PCR-derived dsDNA of indicated lengths at 0.167 μg/ml for 6 h, measured with qPCR.

C, D Elution profile of *in vitro* cGAMP synthesis reaction. 100 nM recombinant human cGAS (ΔN-ter) was incubated with PCR-derived dsDNA (C) or plasmid backbone-derived restriction fragments (D) of indicated lengths at 1 ng/μl in the presence of ATP and GTP for 2 h. Resulting reaction products were purified by column purification. Eluting nucleotides were detected by absorbance at 254 nm.

E Control elution profiles of commercial cGAMP and ATP and GTP stocks used for *in vitro* cGAS activity assays. After column purification, eluting nucleotides were detected by absorbance at 254 nm.

F Area under curves for *in vitro* cGAMP production assay using a circular plasmid, representative of amount of cGAMP generated *in vitro*.

Data information: Data are represented as mean ± SD of biological triplicates in one experiment (A, B) or as mean ± SD of three independent experiments (F). Absorbance values are shown from one representative experiment (C, D). Statistical significance was analyzed using an unpaired, two-tailed *t*-test with Welch's correction for unequal variances. ***$P < 0.001$. The experiments were performed three times.

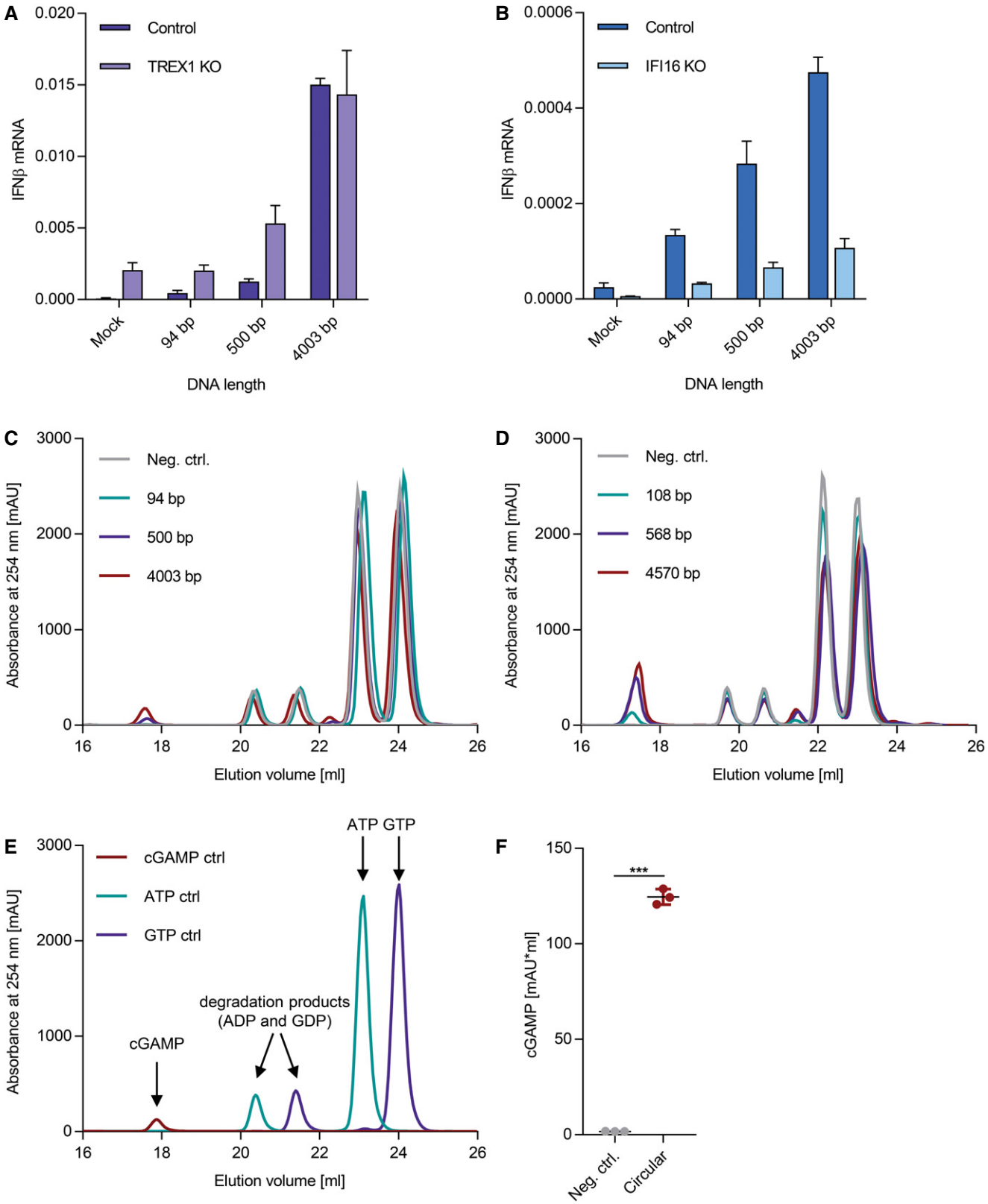


Figure EV4.